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Occurrence of Pesticides Residues in soft tissue of *Buccinum undatum* and *Littorina littorea* from Brackish water and intertidal zone of the lower Atlantic ocean

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Abstract
The occurrence of pesticides residue in *Buccinum undatum* and *Littorina littorea*, the benthic filter feeders at the brackish water of the Ikebiri River and at the intertidal zone of the lower Atlantic Ocean were analyzed. The samples were collected from three stations, two stations along the River (Ngbokolo and Obomikorogbene), and the third (Ebidoughene) experiences occasional Oceanic tides. The samples were collected between January – December, 2016 and a total of sixty samples of each of the specimen were analyzed using high performance liquid chromatography (HPLC model CECIL 1010) to elucidate its concentrations in these gastropods.The concentrations of the pesticide in the matrices ranged between;17.20 – 0.40 µg/gdw (*B. undatum*) and 0.20 – 12.50 µg/gdw (*L. littorea*). The order of concentrations of the pesticides in the gastropods were; in *B.undatum*: Glyphosate formulation > Diazinon > *Paraquat dichloride* > Chloropyrifos, while in *L. littorea*: *Paraquat dichloride* > Glyphosate formulation > Diazinon > Chloropyrifos. Seasonal variation showed that the pesticides concentrations were higher during the dry season than the wet season. Spatial distribution revealed that Ebidoughene > Ngbokolo > Obomikorogbene, an indication that the intertidal zone of the lower Atlantic Ocean had the highest concentration in all the stations examined. The concentrations of the pesticides reported in this investigation were higher than the recommendation limit. The persistent pesticides represent long-term dangers as they bio-magnify up the food-chain and humans are at the top of the food-chain. If the pesticide levels found in water were within the standards set by various regulatory bodies, they are still accounted for a low to high ecotoxicological risk for aquatic organisms, especially algae and macro-invertebrates. Proper measures should be taken while using pesticides, so that their discharge into the water bodies does not endanger aquatic life.

Key words: *Buccinum undatum,Littorina littorea*, Brackish water, Ikebiri River,Atlantic Ocean,Pesticides

Introduction
Nigeria with land mass of 910770km^2 is bordered in the North by Niger and Chad, to the South by the Atlantic Ocean. Three Rivers system drain into the Atlantic ;the Niger (with major tributaries being the Benue, Sokoto,Rima, Kaduna,Gongola, Katsina – Ala,Taraba, Hawal and Anambra),the Atlantic west of the Niger (the Ogun, Oshun, Owena and Benin Rivers) and the Atlantic east of the Niger. All these Rivers empty their content which includes hazardous substances into the Ocean [1]

Open-ocean areas of the Atlantic have remained largely free of human-generated wastes, but increases in marine pollution have been found in poorly mixed coastal waters, especially those located near population and industrial centers and river mouths. Although marine pollution is frequently associated with such activities as ocean dumping, shipping (notably crude oil), and offshore hydrocarbon production, the bulk of it originates from land-based sources such as untreated or poorly treated sewage, industrial waste such as heavy metals, and surface water runoff especially agricultural runoff (fertilizer and pesticides). The most visible effect on polluted waters occurs when pesticides such as *Paraquat dichloride*, endosulfan, DDT and such chemically stable compounds as polychlorinated biphenyls (PCBs), trace levels of which have been measured even in deep-sea Atlantic organisms [2]

The introduction of these harmful compounds into the marine environment appears to be decreasing, but their resistance to chemical breakdown (especially PCBs) and their tendency to accumulate in higher organisms continue to make them a threat to marine life and to humans. Southern Niger Delta is dominated with small seaweeds and microscopic algae that grow on the trunks and roots of the mangroves and on the surface of the mud. This substrate, along with the decaying mangrove leaves, supports a rich and diverse animal community, such as snails, crabs, clams and shrimps are often found in abundance.

Bioaccumulation measurements refer to studies on methods of monitoring the uptake and retention of pollutants like metals or pesticides in organs or tissues of organisms such as fish [2]. Hence [3] and [5] reported that there are aquatic animal species that cannot escape from the detrimental effects of these pollutants.

Pesticides exposure of aquatic life may be a more widespread problem than most people realize, and most pesticide-related aquatic kill go unreported and when, is limited to fish, and the number of fish killed is often underestimated. They are considered systemic toxicants that are known to induce multiple organ damage, even at a low concentration. They are also classified as human carcinogens according to the U.S. Environmental Protection Agency, and the International Agency for Research on Cancer [5]

Organochlorine pesticides are synthetic compounds that are found in nature as a consequence of modern manufacturing processes and agricultural productivity efforts. Because of their stability and hydrophobicity organochlorine pesticides are accumulated in lipids and fatty tissue and their concentration increases up the food chain [6].As regards the fact that snails can accumulate high levels of toxic metals and pesticides they can represent a "critical pathway" for contaminants bio-magnification [7], and that's why permanent control of toxic residues is necessary.

Buccinum undatum and *Littorina littorea* are benthic filter feeders; they are a potential biomonitor of the bioavailability of contaminants in the marine and estuary environment. They serve as food for a large variety of aquatic organism and birds, which in turn make them accessible for human consumption through food chain and eventually pose great health risk [8]

Pesticides residues detected in coastal waters reflect the regional use of pesticides (e.g. DDTs, atrazine, endosulfan, gramoxone), although for more volatile and environmentally persistent compounds (e.g. hexachlorocyclohexane, lindane) long range atmospheric transport also contributes to their far field dispersal in the oceans. Despite the increasing number of pesticide reports in the scientific literature, data on residues are still very scarce for extensive coastal areas in regions of intensive pesticide usage such as the tropics [9]

Pesticides usage in Niger Delta ecological zone is very high and metal pollution is on increase [10]. Accumulation of these pesticides in *B. undatum* and *L. littorea* has not been seriously investigated in this region, and the world at large. Hence, the bioaccumulation database of this investigation might be very useful for pesticides and metal monitoring programme in brackish water and marine environment, which are very rich with aquatic organisms.

Materials and Methods

Study area: The Ikebiri River drains into the Atlantic Ocean at Ebidoughene (Station 1). The stations submerged with water during high tide and exposed to the air during low tide. The area is sandy and has rocky cliffs. Upstream to this station are the other stations; Ngbokolo (Station 2) and Obomikorogbene (station 3) (Figure 1). These stations do not experience tide and the water are slightly stratified, the saline water circulates in at the bottom, mixes with fresh water, and then flows out at the top (salinity thus increases with depth and out toward the ocean).

Agricultural activities, domestic and industrial effluents containing various organic and inorganic pollutants, like heavy metals, pesticides, oils, and fertilizers etc are regularly discharged into this river consciously and unconsciously, thereby affecting its water quality.

Samples collection: Samples were collected between January and December, 2016. Five samples each of common whelk and *L. littorea* of relatively equal size and weight were collected at night (they are more of nocturnal than diurnal) from different stations (Figure 1) and transported to the laboratory in a well ventilated container. The sample stations were visited monthly for 12 months and a total of sixty samples of each of the specimen were analyzed for the present of pesticides

Sample preparation: In the laboratory, the body tissues were removed, washed in distilled water and dried separately in oven at about 110ºC to constant weight. After complete drying the tissues were powdered in mortar and pastel and stored separately by labeling the specimen with date and species name.

Sample extraction and analysis: Pesticides were extracted from the residue using redistilled mixture of dichloromethane and n – hexane in a ratio 2: 3. The samples were cleaned until it became a clear, colourless and concentrated to the final volume between 0.25 ml and 1 ml and ready for high performance liquid chromatography analysis.

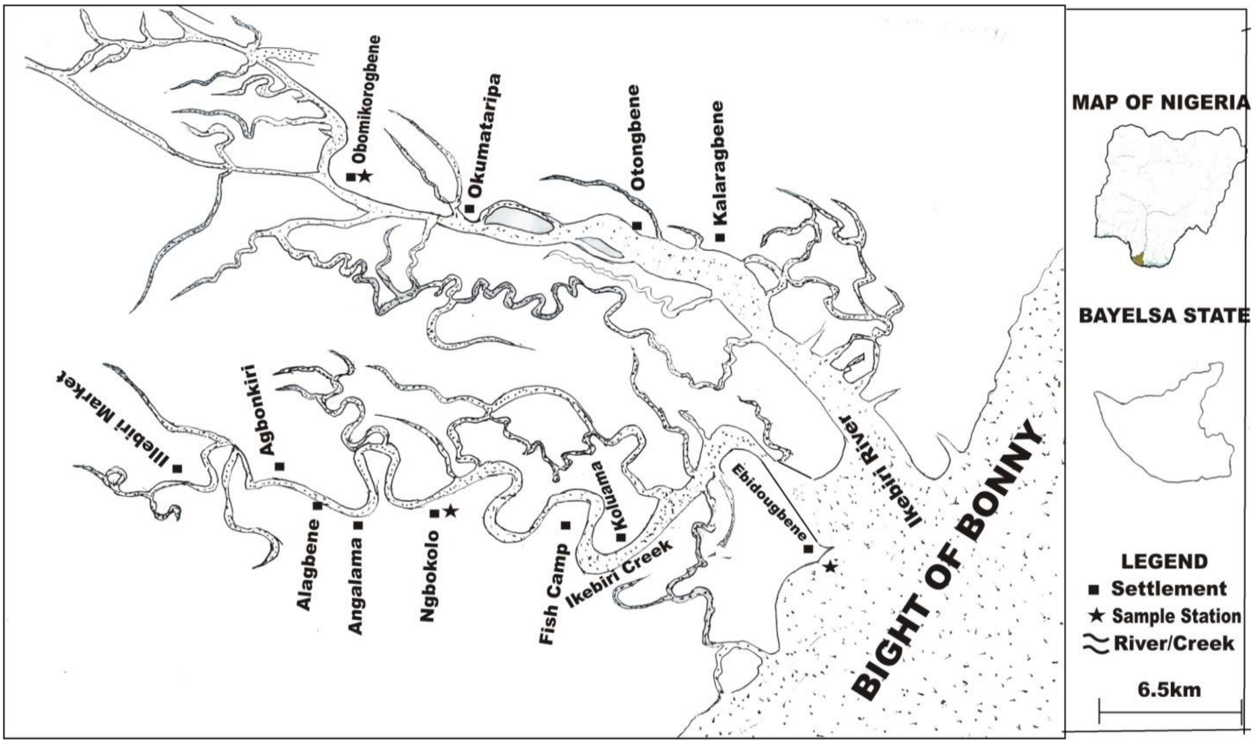

Figure: Map of Ikebiri River showing sampling stations. Ebidougbene station is at the point where the river empties its contents into the Atlantic Ocean

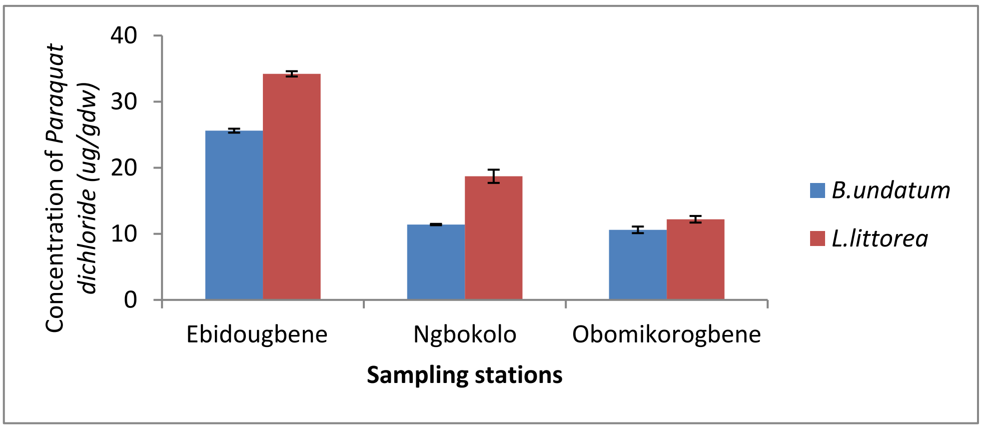

Figure 2: Concentrations of *Paraquat dichloride* in *B.undatum* and *L.littorea* from Ikebiri River and Intertidal zone of Atlantic Ocean

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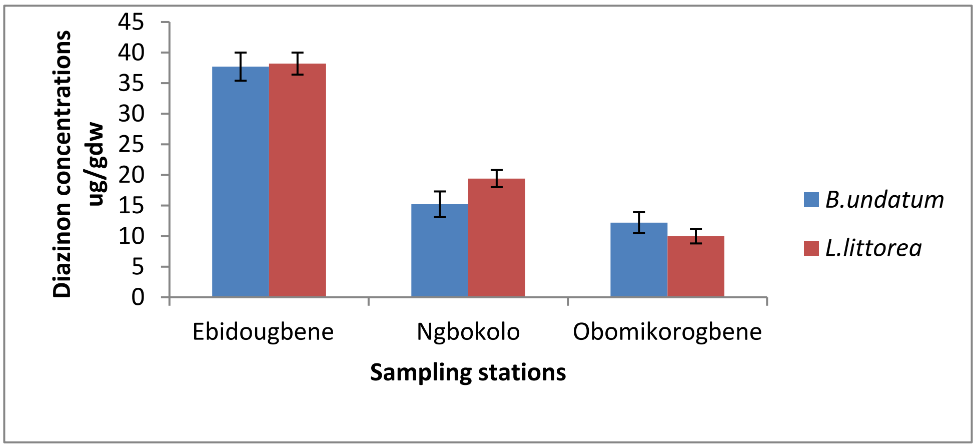

Figure 3: Concentrations of Diazinon in *B.undatum* and *L.littorea* from Ikebiri River and Intertidal zone of Atlantic Ocean

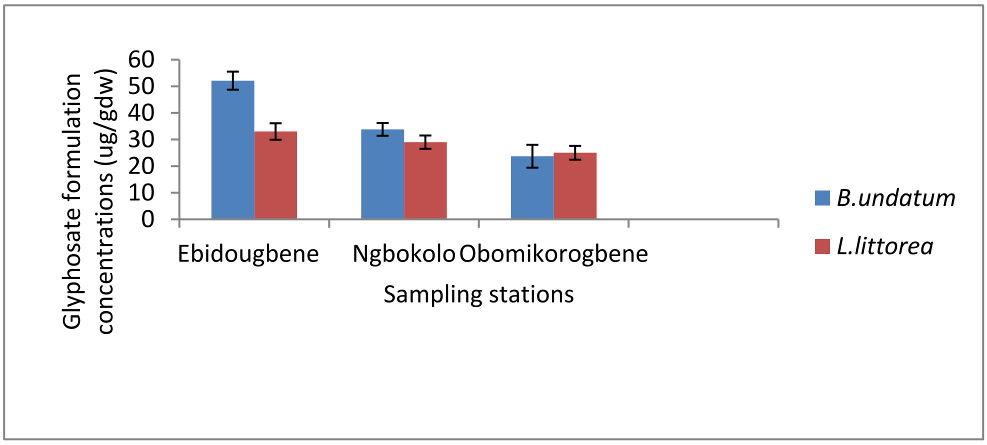

Figure 4: Concentrations of Glyphosate formulation in *B.undatum* and *L.littorea* from Ikebiri River and Intertidal zone of Atlantic Ocean

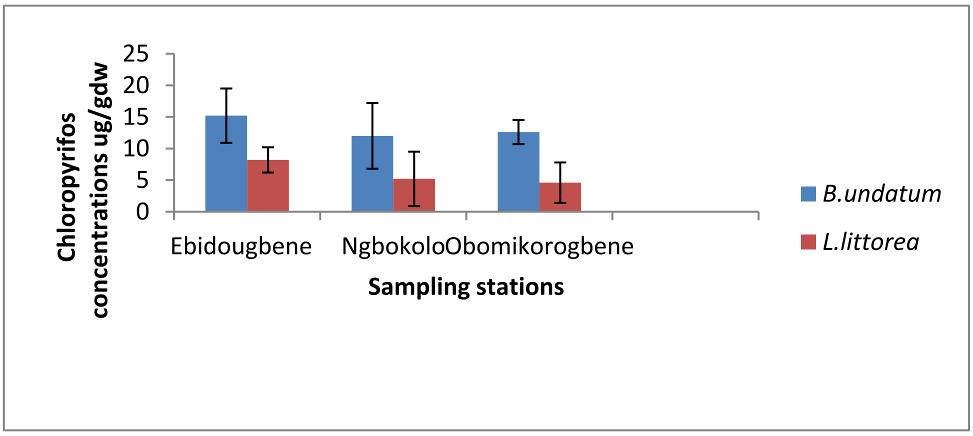

Figure 5: Concentrations of Chloropyrifos in *B.undatum* and *L.littorea* from Ikebiri River and Intertidal zone of Atlantic Ocean

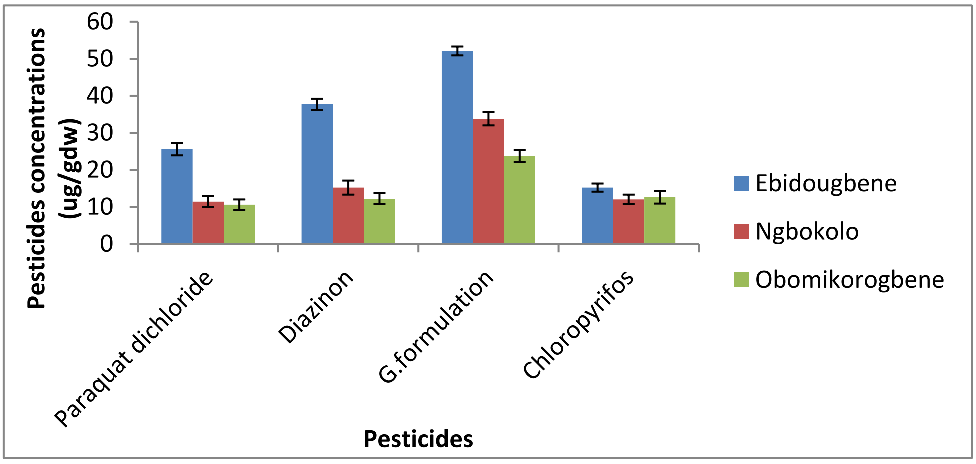

Figure 6: Comparison of the concentrations of pesticides residue in the soft tissue of *B.undatum* from Ikebiri river and the inter- tidal zone of Atlantic Ocean.

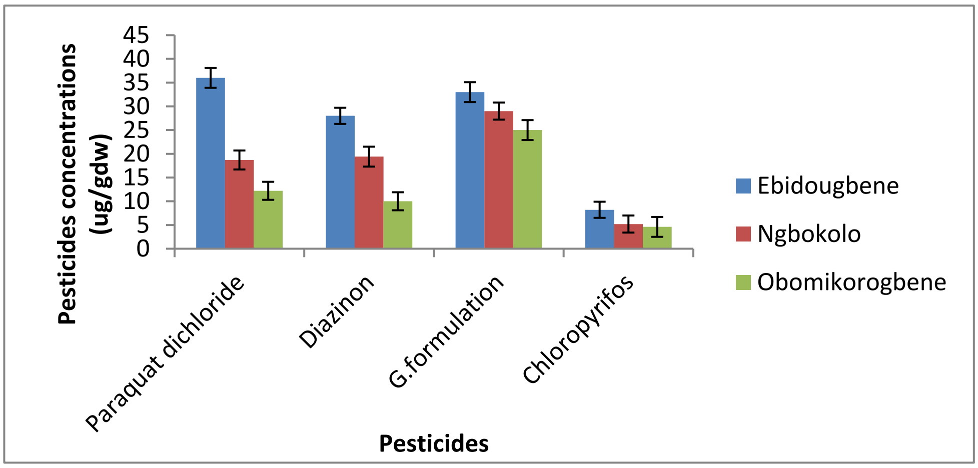

Figure 7: Comparison of the concentrations of pesticides residue in the soft tissue of *L. littorea* from Ikebiri River and the intertidal zone of Atlantic Ocean.

Table 1: Pesticides concentrations (µg/gdw) during the dry and wet seasons in soft tissue of *L. littorea* from brackish water and intertidal zone of the lower Atlantic Ocean, sampled monthly from January to December, 2016. Means are based on the monthly observations.

	Paraquat dichloride		Diazinon		Glyphosate formulation		Chloropyrifos	
	Mean ± SE	Range	Mean ± SE	Range	Mean ± SE	Range	Mean ± SE	Range
Dry season								
Ebidoughene	12.50± 0.3	17.20 –5.30	3.30 ± 0.10	7.00 – 1.40	9.00±0.10	9.20 – 3.50	2.10 ± 0.2	5.20 – 0.80
Ngbokolo	5.20± 0.1	9.20 – 2.10	2.80 ± 0.20	4.40 – 1.20	5.60± 0.30	6.10 - 1.00	1.20 ± 0.1	3.20 – 0.50
Obomikorogbene	4.70 ± 0.2	6.40 – 1.60	2.10 ± 0.10	3.90 – 1.00	5.30±0.40	7.20 – 1.20	0.80 ± 0.1	1.20 – 0.40
Wet season								
Ebidoughene	6.20± 0.1	7.40 –2.10	1.60 ± 0.10	3.20 – 1.00	6.20±0.20	8.20 – 1.20	1.60 ± 0.2	2.10 – 0.60
Ngbokolo	4.00± 0.1	5.20 – 2.10	1.20 ± 0.20	1.80 – 0.20	3.10± 0.10	5.20 - 1.00	1.20 ± 0.1	1.80 – 0.80
Obomikorogbene	2.70 ± 0.2	4.40 – 1.60	1.00 ± 0.30	1.30 – 0.20	1.20±0.10	1.90 – 0.50	0.40 ± 0.1	0.60 – 0.20

Table 2: Pesticides concentrations (µg/gdw) during the dry and wet seasons in soft tissue of *B.undatum* from brackish water and intertidal zone of the lower Atlantic Ocean, sampled monthly from January to December, 2016. Means are based on the monthly observations.

	Paraquat dichloride		Diazinon		Glyphosate formulation		Chloropyrifos	
	Mean ± SE	Range	Mean ± SE	Range	Mean ± SE	Range	Mean ± SE	Range
Dry season								
Ebidoughene	17.20± 1.2	21.00 –7.00	5.20 ± 0.30	7.00 – 4.20	12.20±0.30	14.40 –10.50	3.40 ± 0.6	5.20 – 0.80
Ngbokolo	12.10± 0.6	16.00 – 3.00	3.20 ± 0.10	5 .00 – 2.10	10.20±0.10	10.10 - 1.00	1.90 ± 0.2	3.20 – 0.50
Obomikorogbene	7.20 ± 0.1	11.50 –5.00	3.10 ± 0.10	5.00 – 2.00	6.10±0.20	7.20 – 1.20	1.20 ± 0.3	1.20 – 0.40
Wet season								
Ebidoughene	13.00± 0.2	17.20 –7.40	4.40 ± 0.20	6.00 – 2.70	6.20±0.20	8.20 – 1.20	2.20 ± 0.1	3.20 – 1.80
Ngbokolo	8.00± 0.6	10.20 – 5.10	3.10 ± 0.10	4.30 – 1.20	3.10± 0.10	5.20 - 2.00	1.80 ± 0.2	2.70 – 1.10
Obomikorogbene	5.10 ± 0.1	7.40 – 3.10	1.60 ± 0.20	2.80 – 0.90	2.20±0.02	4.00 – 0.80	1.20 ± 0.1	1.80 – 0.40

Data analysis

The data were summarized separately for each sampled station using Descriptive statistics (means, range, and histogram). Statistical differences between the seasons were analysed using Student's t test, while the differences between the marine and brackish stations were analysed using oneway analysis of variance with confidence range of $p<0.05$ with SPSS (16.0 version), SPSS Inc, USA.

Results

The results of this study showed the presence of organochlorine (OC) and organophosphorus (OP) pesticides in two most common gastropods in Ikebiri River and the Inter-tidal zone of the Atlantic Ocean. Though, there is no clear distinction in the concentrations of these pesticides in these two environments ($p > 0.05$), but there was a slight variation. The seasonal concentrations of various pesticides in the soft tissues of *L. littorea* and *B.undatum* from Brackish water of the Ikebiri river and the marine environment are shown in Table 1 and 2, with further illustration in Figure 2- 6.

All the pesticides were present in all the samples analyzed with glyphosate formulation having the highest concentrations (52.10 ± 2.3µg/gdw) and were recorded in *B.undatum* at Ebidougbene station, while Chloropyrifos was the least pesticide recorded (4.6±0.30µg/gdw), and was observed in *L. littorea* at Obomikorogbene station.

B.undatum from the stations had higher affinity for this pesticides accumulation than the *L. littorea* (Figure 2-5). The order of concentrations of the pesticides in the gastropods are; in *B.undatum*: Glyphosate formulation > Diazinon > *Paraquat dichloride* > Chloropyrifos (Fig.6), while in *L. littorea* the order was: *Paraquat dichloride* > Glyphosate formulation > Diazinon > Chloropyrifos (Fig.7). The concentration of the pesticides in the two environments did not show any significant variation ($p > 0.5$)

Seasonal variation showed that the pesticides concentrations were higher during the dry season than the wet season in the gastropods examined. In *L. littorea*, during the dry season, the pesticides concentrations range between 12.50– 0.40 µg/gdw, while in the wet season the range was between 0.20 – 6.20 µg/gdw (Table 1). In *B.undatum,* the range in the dry season was between 0.40 – 17.20 µg/gdw, while the wet season ranged between 0.40 – 13.00 µg/gdw (Table 2).

Spatial and ecological distribution revealed that; in *B.undatum*, Ebidoughene had the highest pesticides concentration, observed in Glyphosate formulation (52.10µg/gdw) and the least recorded at Ngbokolo in Chloropyrifos (12.00 µg/gdw); in *L. littorea*, *Paraquat dichloride* was the highest pesticide residue observed (36.00 µg/gdw) at Ebidoughene, while Chloropyrifos (4.60 µg/gdw), the least and was recorded at Ngbokolo (Figure 6and 7)

Seasonal variation showed that the pesticides concentrations were higher during the dry season than the wet season in the gastropods examined. In *L. littorea*, during the dry season, the pesticides concentrations range between 12.50– 0.40 µg/gdw, while in the wet season the range was between 0.20 – 6.20 µg/gdw (Table 1). In *B.undatum,* the range in the dry season was between 0.40 – 17.20 µg/gdw,

while the wet season ranged between 0.40 – 13.00 μg/gdw (Table 2).

Discussion

The concentrations of pesticides in estuarine and intertidal zone of Atlantic Ocean were investigated in *B.undatum* and *L. littorea* for a period of twelve months. Though, there is no clear distinction in the concentrations of these pesticides in these two environments, their concentrations in these gastropods were high, with the organochlorine pesticides having higher concentrations than organophosphate pesticides. The high concentrations observed in OC pesticides (glyphosate formulation and *Paraquat dichloride*) could be attributed the fact that OCs are resistant to microbial and photolytic degradation, and are therefore persistent in the environment (soils and water), and can be easily taken up by the detritus feeders, while the OPs are readily de-activated and degraded by microbial activities [11,12]. The high concentrations of OCs may have far reaching effects on non-target aquatic organism [13]. Roundup, a globally used herbicide caused high mortality of aquatic organism under natural conditions in an outdoor [13]

The study also revealed that the concentrations of the pesticides in the estuary and marine environment peak in dry season and low in wet season. These seasonal patterns in pesticides concentrations are likely related to differences in field application. This is due to the mangrove and mash nature of the environment that makes agriculture activities impossible during the wet season. People in this region cultivate crops where these pesticides are use for crops protection and weeds control during the dry season.

The concentrations of the pesticides were higher in *B.undatum* than in *L. littorea*. The variation could be attributed to the size of the gastropods, fatty nature and their different living habits. According to [14] the accumulation of organic contaminants in the tissue of aquatic organisms is a complex function of the physicochemical properties of the contaminants, its distributions in the aquatic system, the feeding, behaviour and metabolism of the aquatic organism and their water uptake solubility [9,15]. For example, Clomazone, a popular herbicide, is particularly water soluble; a property that increases its likelihood of contaminating surface, groundwater and aquatic organism. The hydrophilic or lipophobic nature of this pesticide makes it less available in the fatty tissues of an organism [15]

The intertidal zone of the lower Atlantic Ocean had the highest concentration in all the stations examined. The factor that might have influenced this could be attributed to the alternating submergence and exposure and the geological nature of shorelines and near shore bottoms. Other factor that might play a role in pesticide persistence is climate characteristics. Studies in the Arctic have shown that insecticides and herbicides persist 3 to 8 times longer in cold climates than in temperate ones [16]. Also, low concentrations of pesticides in estuaries could also be as a result of dilution caused by degradation and sorption to suspended matter and sediments [17]. Similar observation was reported by in fish samples from the marine environment and tambak, a brackish river in Indonesia. The marine fish showed a higher value of 2, 4 D-isopropyl ester residues than the tambak samples, that is brackish water [18]

The persistent pesticides represent long-term dangers as they bio-magnify up the food-chain and humans are at the top of the food-chain. If the pesticide levels found in water were within the standards set by various regulatory bodies, they are still accounted for a low to high ecotoxicological risk for aquatic organisms, especially algae and macro-invertebrates. Proper measures should be taken while using pesticides, so that their discharge into the water bodies does not endanger aquatic life.

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